

Supporting Information

Integrating Molecular Networking and ¹H NMR Spectroscopy for Isolation of Bioactive Metabolites from the Persian Gulf Sponge *Axinella sinoxea*

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Table S1. Putative identification of known compounds in the global MN of the crude MeOH extract and CHCl₃ subextract of *Axinella sinoxea*.

Cluster A and A1								
No.	<i>t_R</i> (min)	Parent mass <i>m/z</i>	Putative ID	Molecular formula of the <i>m/z</i> [M + H] ⁺	Δ ppm	Key MS ² fragments	LoA*	Reference
1 ^b	8.3	468.321	1- <i>O</i> -pentadecyl- <i>sn</i> -glycero-3-phosphocholine	C ₂₃ H ₅₁ NO ₆ P	2.1	450.4125; 285.0731; 184.0750; 104.1051	3	1
3 ^a	8.2	480.321	1-(1 <i>Z</i> -hexadecenyl)- <i>sn</i> -glycero-3-phosphocholine	C ₂₄ H ₅₁ NO ₆ P	1.1	462.3144; 297.2121; 184.3345; 104.3651	3	2
4 ^a	9.0	482.339	1- <i>O</i> -hexadecyl- <i>sn</i> -glycero-3-phosphocholine	C ₂₄ H ₅₃ NO ₆ P	1.2	464.2720; 299.2960; 184.1212; 104.2348	5	3
5 ^b	8.7	494.309	1-(1 <i>Z</i> -heptadecenyl)-glycero-3-phosphocholine	C ₂₅ H ₅₃ NO ₆ P	-1.5	476.5814; 311.5471; 184.4714; 104.7451	2	4
6 ^a	8.7	496.314	1- <i>O</i> -heptadecyl- <i>sn</i> -glycero-3-phosphocholine	C ₂₅ H ₅₅ NO ₆ P	0.8	478.3310; 313.2881; 184.3414; 104.3257	3	5
7 ^b	11.4	508.352	1-(1 <i>Z</i> -octadecenyl)- <i>sn</i> -glycero-3-phosphocholine	C ₂₆ H ₅₅ NO ₆ P	-1.1	490.8764; 325.4761; 184.5487; 104.5541	2	2
8 ^b	10.1	510.369	1- <i>O</i> -octadecyl- <i>sn</i> -glycero-3-phosphocholine	C ₂₆ H ₅₇ NO ₆ P	2.1	492.4541; 327.5151; 184.3561; 104.3821	3	6
9 ^b	8.4	512.374	1- <i>O</i> -(2-methoxyhexadecyl)- <i>sn</i> -glycero-3-phosphocholine	C ₂₅ H ₅₅ NO ₇ P	3.2	494.3871; 329.4542; 184.3320; 104.3224	2	7
10 ^a	9.9	524.349	1- <i>O</i> -octadecanoyl- <i>sn</i> -glycero-3-phosphocholine	C ₂₆ H ₅₅ NO ₇ P	0.9	506.3620; 341.3040; 184.0740; 104.1070	5	8
11 ^b	10.5	538.347	1- <i>O</i> -nonadecanoyl- <i>sn</i> -glycero-3-phosphocholine	C ₂₇ H ₅₇ NO ₇ P	1.3	520.3945; 510.3910; 355.4573; 184.0531; 104.1273	3	8
12 ^a	9.0	552.379	1-arachidoyl-2-hydroxy- <i>sn</i> -glycero-3-phosphocholine	C ₂₈ H ₅₉ NO ₇ P	2.2	534.3920; 369.3310; 184.1312; 104.1136	3	9
Cluster B and B1								
No.	<i>t_R</i> (min)	Parent mass <i>m/z</i>	Putative ID	Molecular formula of the <i>m/z</i> [M + H] ⁺	Δ ppm	Key MS ² fragments	LoA*	Reference

1 ^b	7.4	385.428	(22 <i>E</i>)-3 β -hydroxy-26,27-bisnorcholesta-5,22-diene-7,24-dione	C ₂₅ H ₃₇ O ₃	1.7	367.2640; 349.2560; 315.2370; 287.2001; 251.1752; 157.1041; 99.0800	2	10
2 ^b	7.8	387.264	3 β -hydroxy-26,27-bisnorcholesta-5-ene-7,24-dione	C ₂₅ H ₃₉ O ₃	1.2	369.2454; 351.2670; 269.2850; 255.2280; 157.1012; 175.1290, 99.1812	2	10
3 ^b	10.1	399.301	(22 <i>E</i>)-3 β -hydroxycholesta-5,22-dien-24-one	C ₂₇ H ₄₃ O ₂	-0.6	381.3201; 363.3225; 283.2678; 269.2138; 173.1012; 111.1167; 69.0690	2	10
4 ^a	12.1	401.316	7-ketocholesterol	C ₂₇ H ₄₅ O ₂	0.6	383.3300; 365.3230; 271.2050; 253.1241; 175.1108; 159.1214; 81.05415	3	11
5 ^b	11.6	413.316	(22 <i>E</i>)-3 β -hydroxycholesta-5,22-diene-7,24-dione	C ₂₇ H ₄₁ O ₃	-0.6	395.4581; 377.3200; 315.2341; 291.8412; 175.1115; 121.3245; 83.0530	5	10
6 ^b	9.1	415.297	3 β -hydroxycholesta-5-ene-7,24-dione	C ₂₇ H ₄₃ O ₃	0.3	397.3110; 379.3145; 361.2912; 293.2341; 203.5412; 127.1218; 109.5471;	5	10

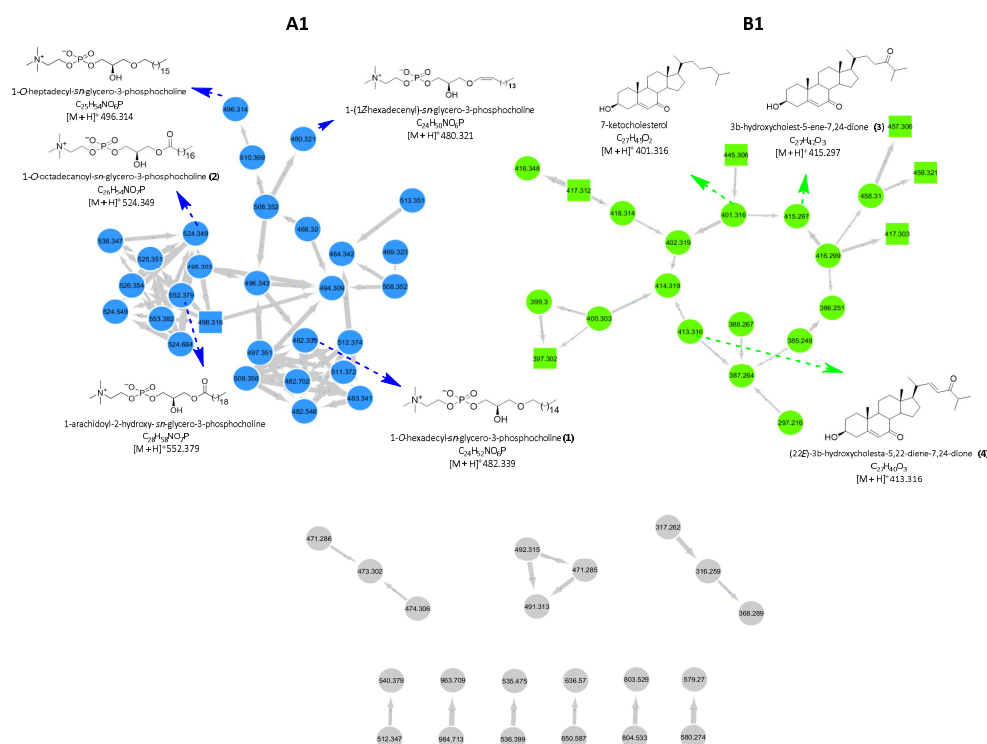
*: LoA (Level of Assignment); 1: Accurate mass matched to database-Tentative assignment, 2: Accurate mass matched to database and tandem MS spectrum matched to *in silico* fragmentation pattern, 3: Tandem MS spectrum matched to database or literature, 4: RT matched to standard compound, 5: MS/MS spectrum matched to standard compound.

^a: Annotation via automated dereplication; ^b: Annotation via manual dereplication

Automated dereplication was performed on GNPS platform. Manual dereplication was performed considering the parent mass, biological source, retention time, elemental composition analysis, and predicated fragmentation patterns.

The MS² fragmentation pattern of a molecule was predicated on the Competitive Fragmentation Modeling for Metabolite Identification (CFM-ID) platform (<http://cfmid.wishartlab.com>) and compared with our experimental data.

Figure S1. Global molecular networking of the CHCl₃ subextract (KC) of *Axinella sinoxea*.



Blue nodes represent phospholipids and green nodes represent steroids. The thickness of the edges indicates the similarity of the nodes. The numbers within the nodes represent parent ions. Square nodes represent putatively new compounds. Gray nodes could not be annotated to any known chemical classes either by automated or manual dereplication. All molecules from clusters **A1** and **B1** were annotated to be the same as those in the global MN of the crude extract of *Axinella sinoxea* (Table S1).

Figure S2. ^1H NMR spectrum of the crude extract of *Axinella sinoxea* (600 MHz, CD_3OD)

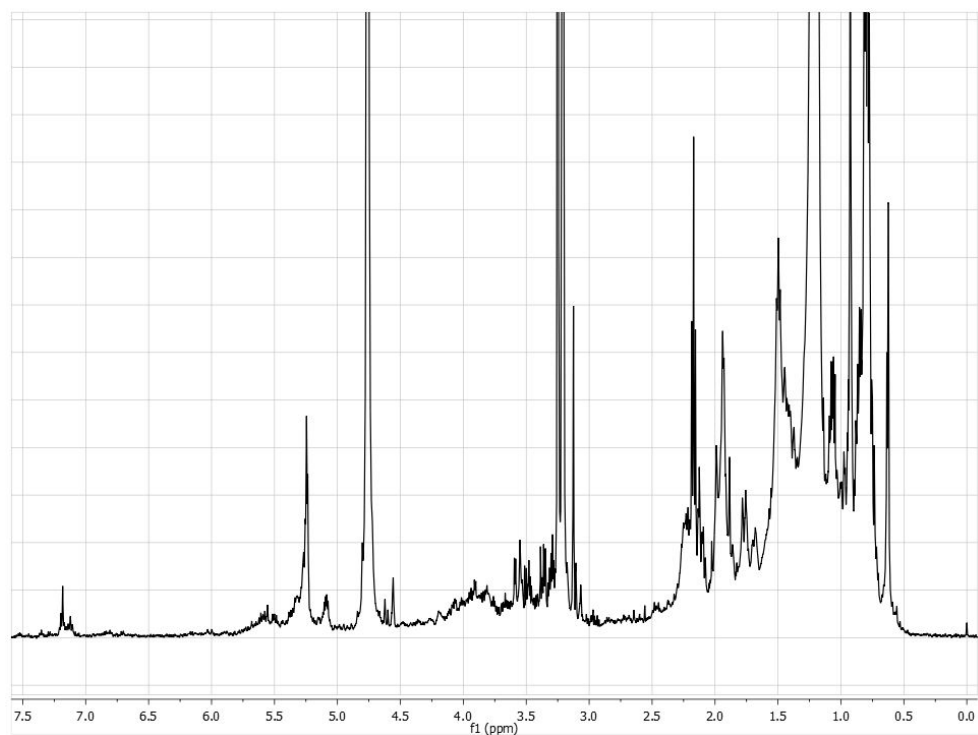


Figure S3. ^1H NMR spectrum of the CHCl_3 subextract (KC) of *Axinella sinoxea*. (600 MHz, CD_3OD)

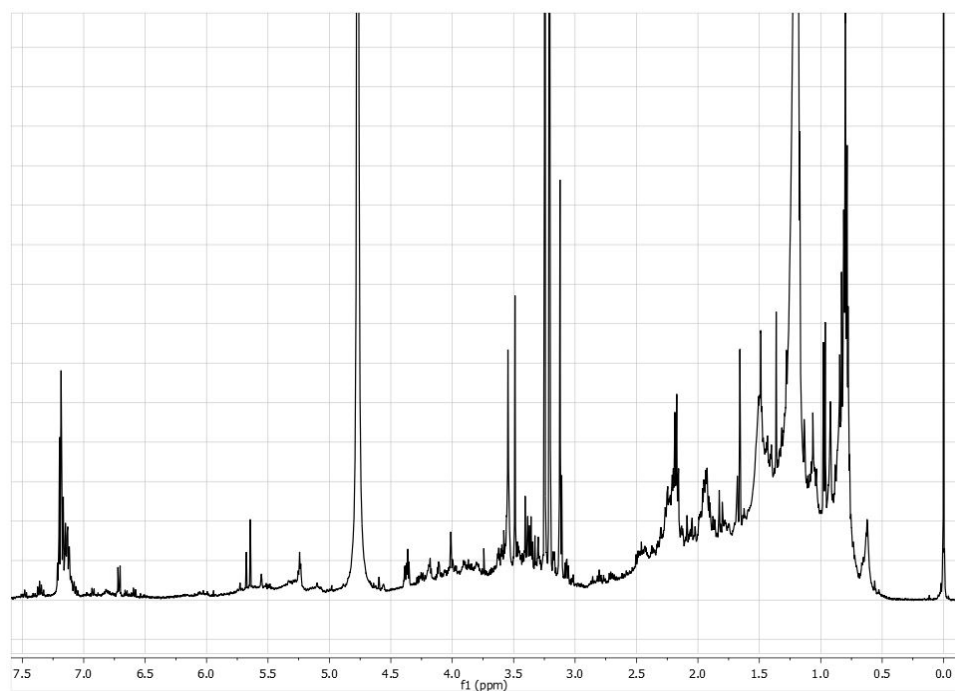


Figure S4. ^1H NMR spectrum of compound **1** (600 MHz, CD_3OD).

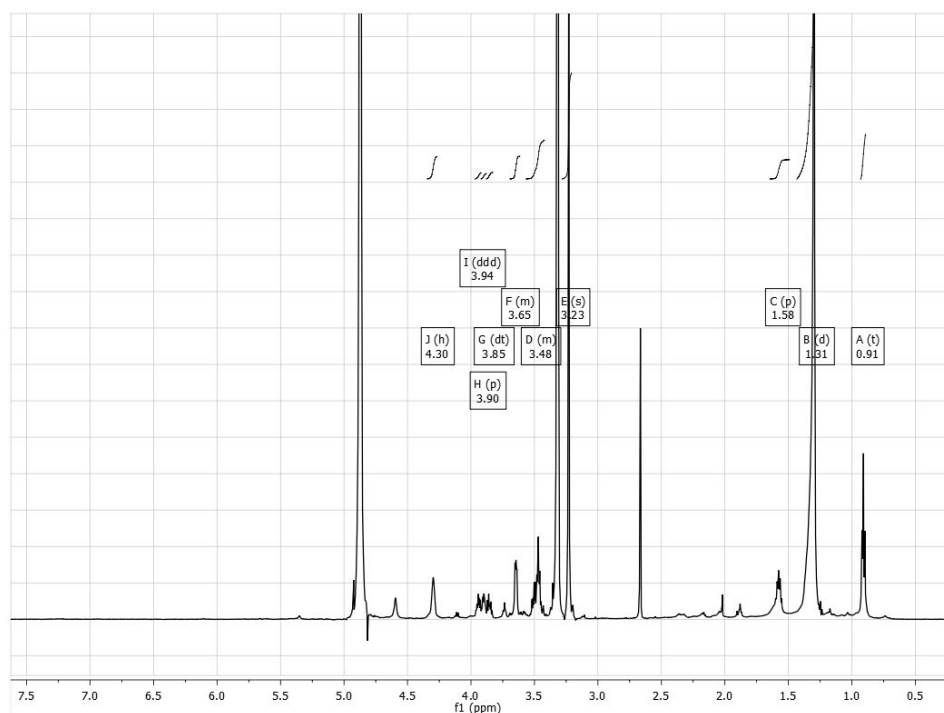


Figure S5. ^{13}C NMR spectrum of compound **1** (150 MHz, CD_3OD).

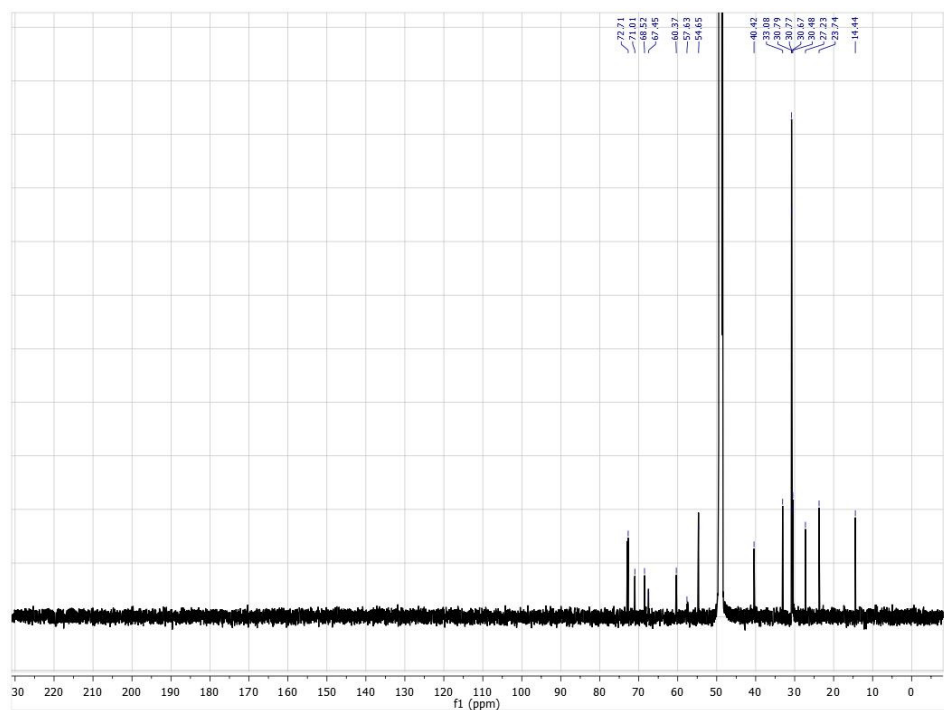


Figure S6. HR-ESIMS spectrum of compound **1**.

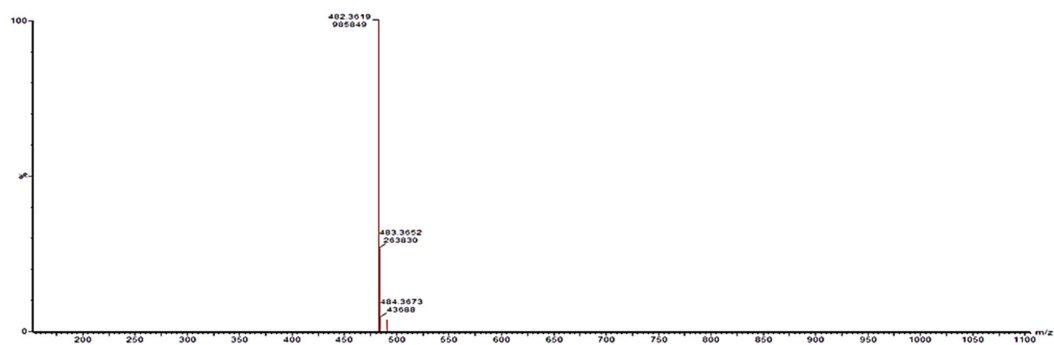


Figure S7. ^1H NMR spectrum of compound **2** (600 MHz, CD_3OD).

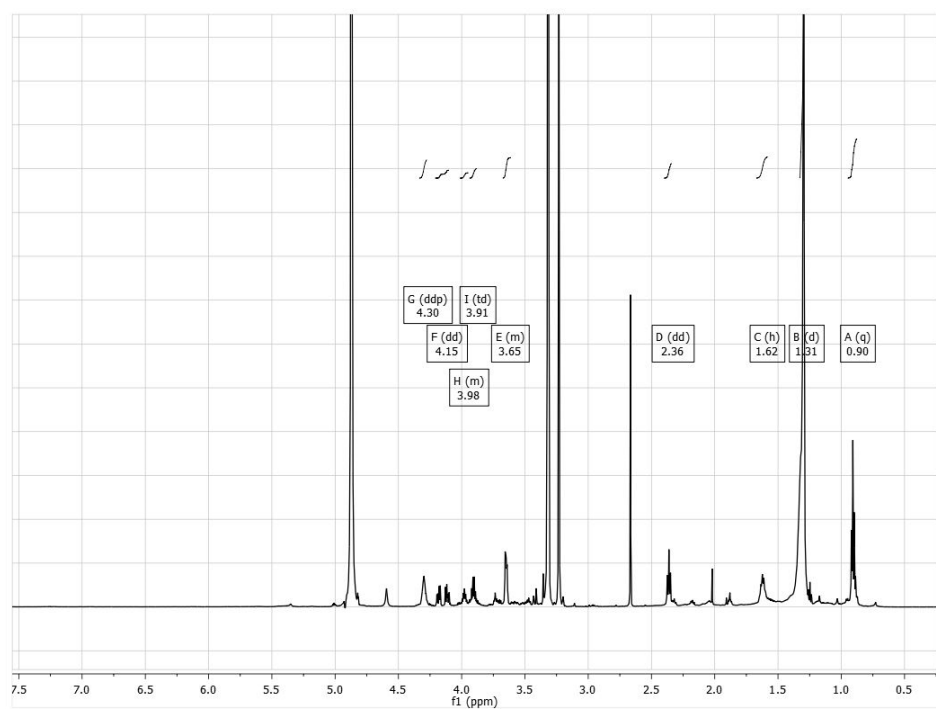


Figure S8. ^{13}C NMR* spectrum of compound **2** (150 MHz, CD_3OD).

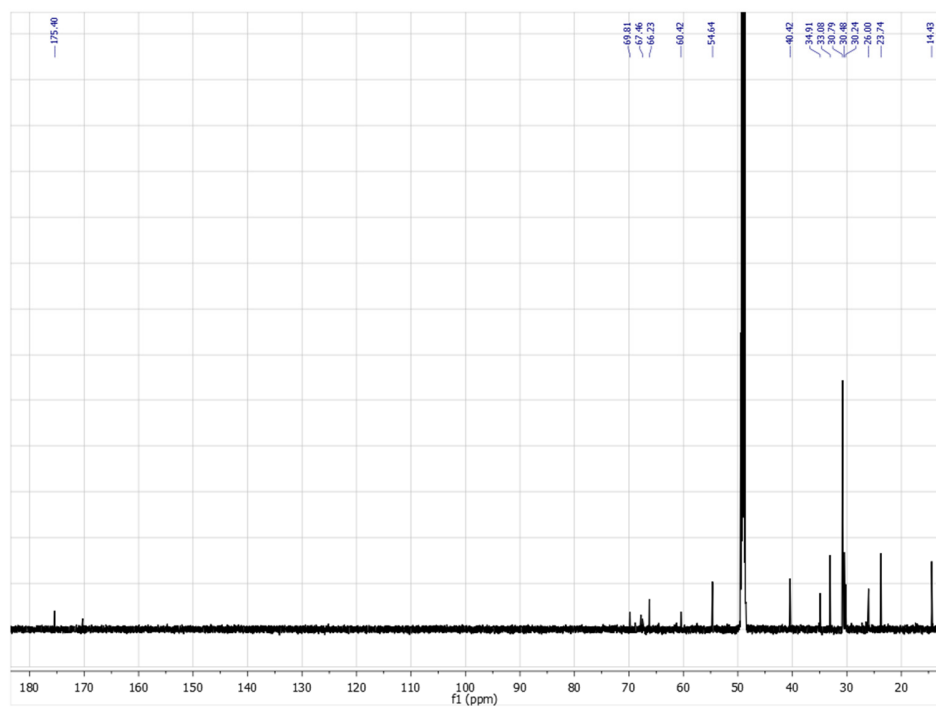


Figure S9. HR-ESIMS spectrum of compound **2**.

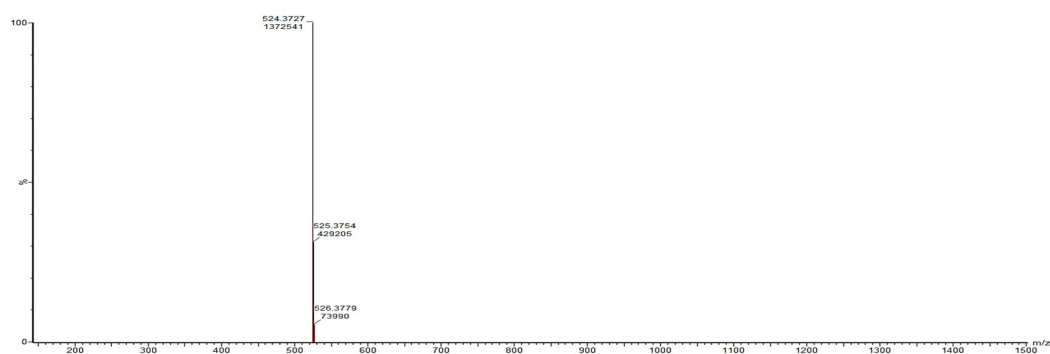


Figure S10. ^1H NMR spectrum of compound **3** (600 MHz, CHCl_3).

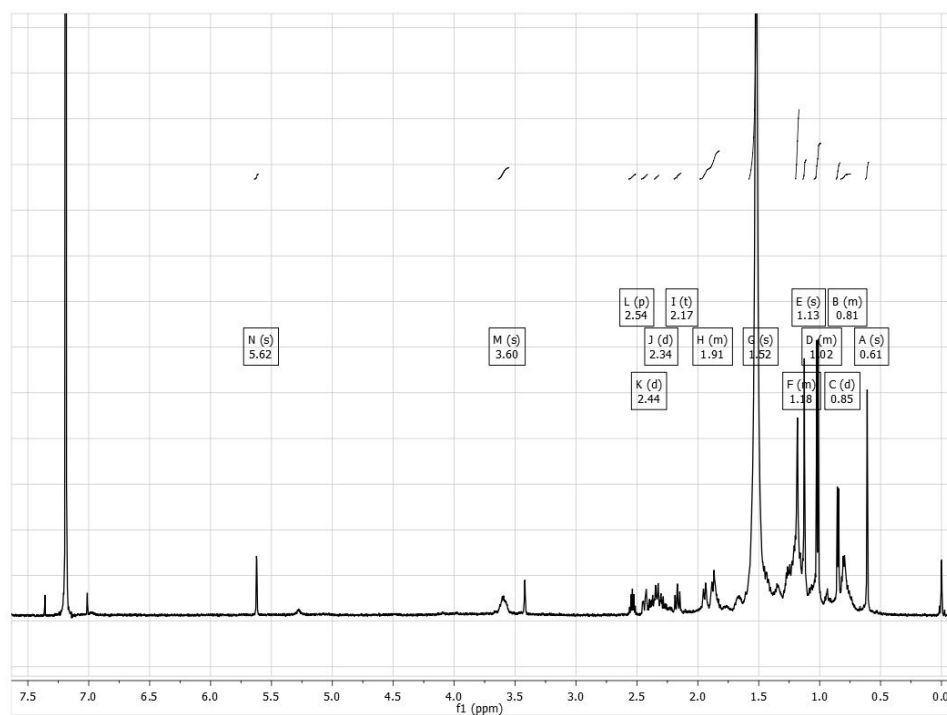


Figure S11. ^{13}C NMR spectrum of compound **3** (150 MHz, CHCl_3).

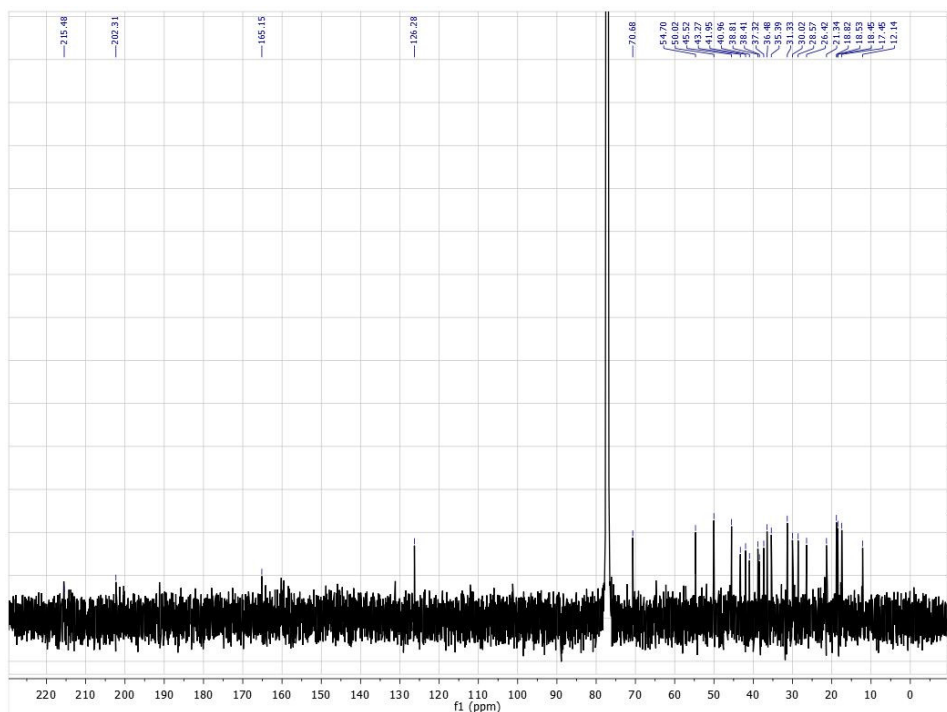


Figure S12. HR-ESIMS spectrum of compound **3**.



Figure S13. ^1H NMR spectrum of compound **4** (600 MHz, CHCl_3).

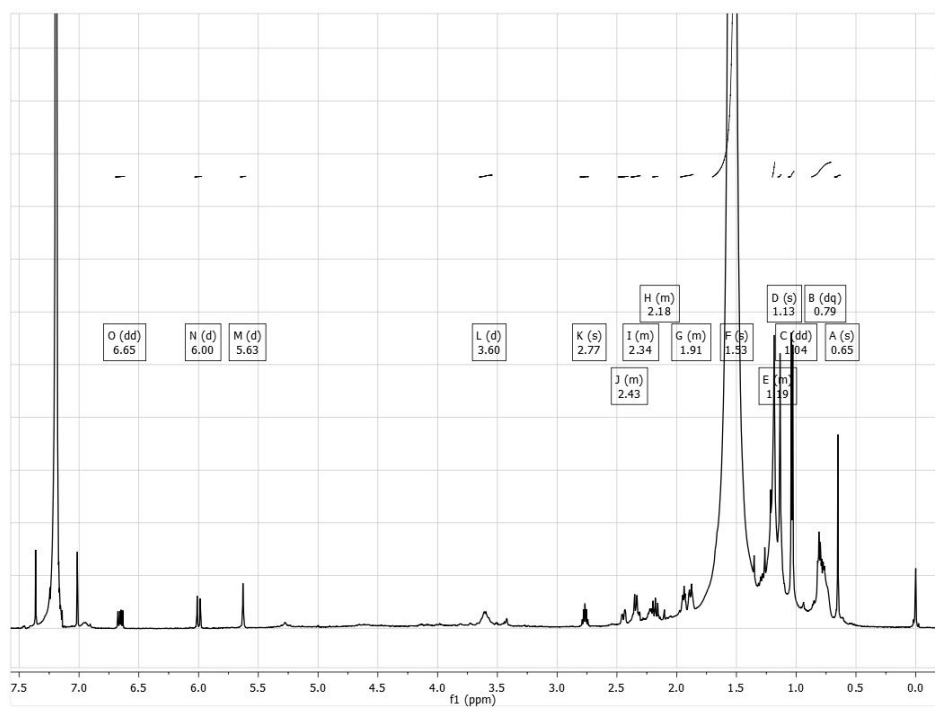


Figure S14. HMBC spectrum of compound **4** (600 MHz, CHCl_3).

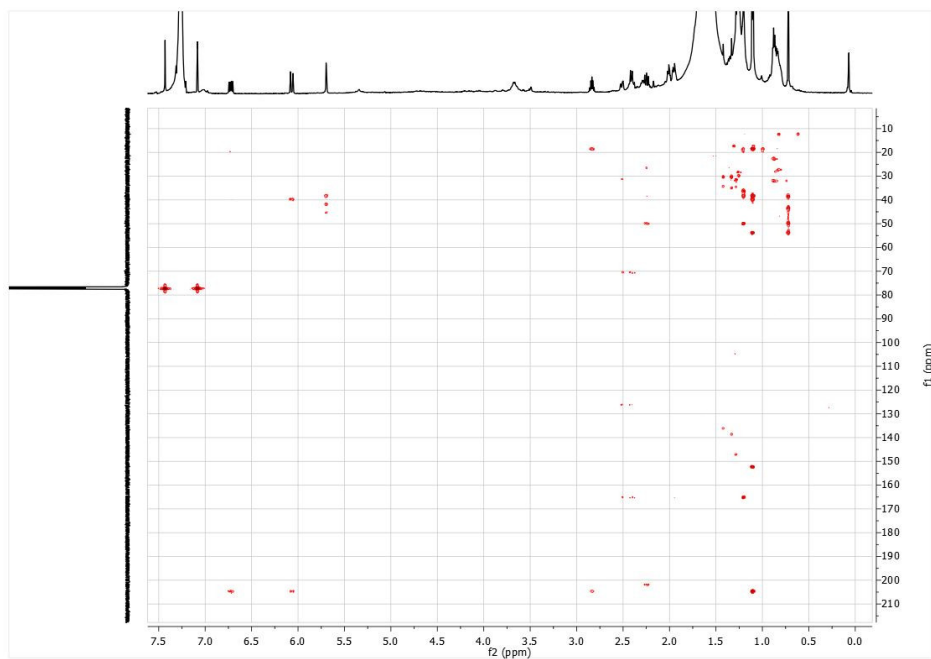


Figure S15. HR-ESIMS spectrum of compound **4**.

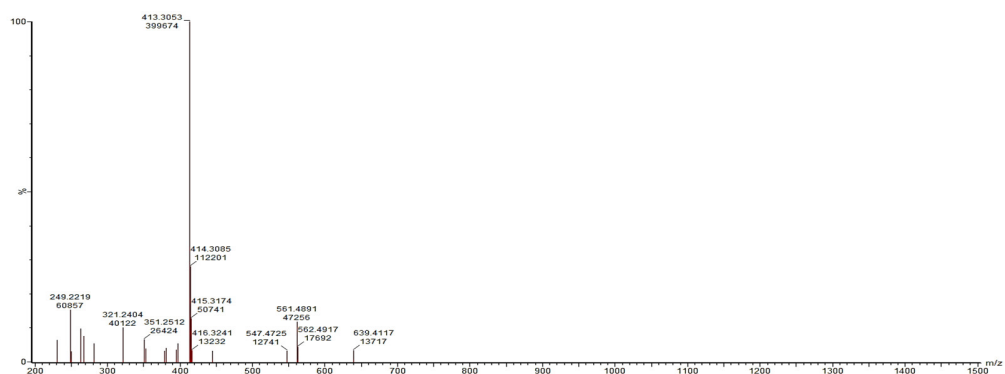


Figure S16. ^1H NMR spectrum of compound **5** (600 MHz, CD_3OD).

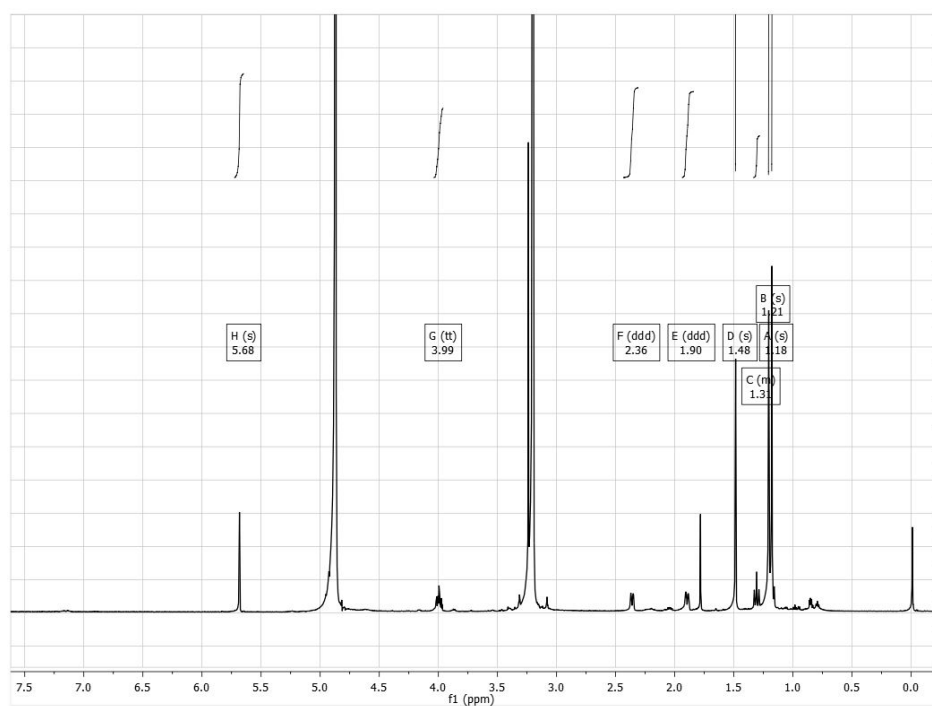


Figure S17. ^{13}C NMR spectrum of compound **5** (150 MHz, CD_3OD).

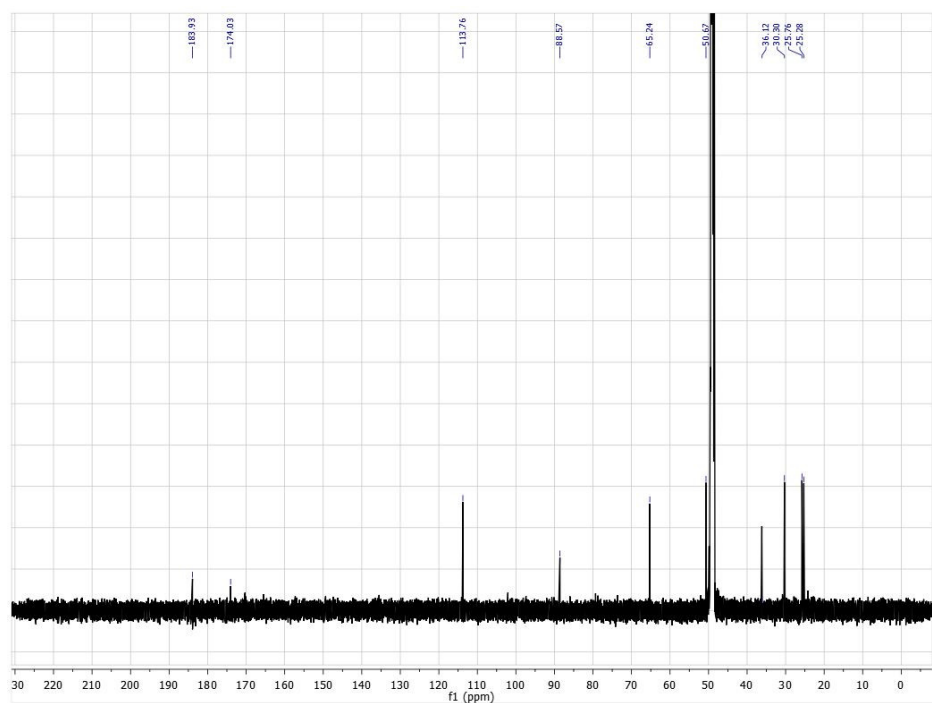


Figure S18. HR-ESIMS spectrum of compound **5**.

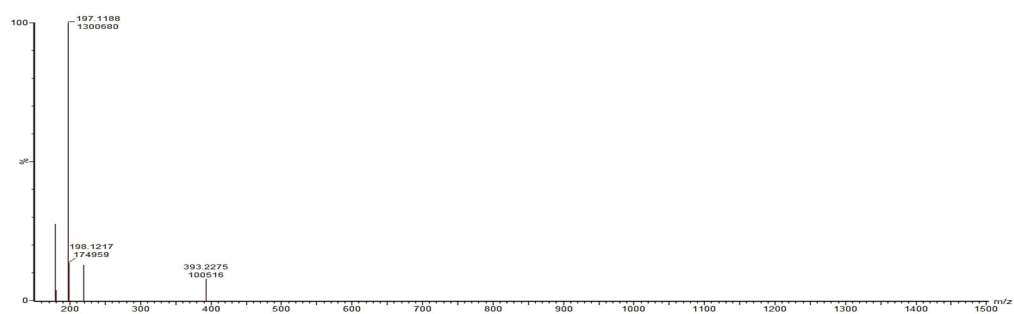


Figure S19. ^1H NMR spectrum of compound **6** (600 MHz, CD_3OD).

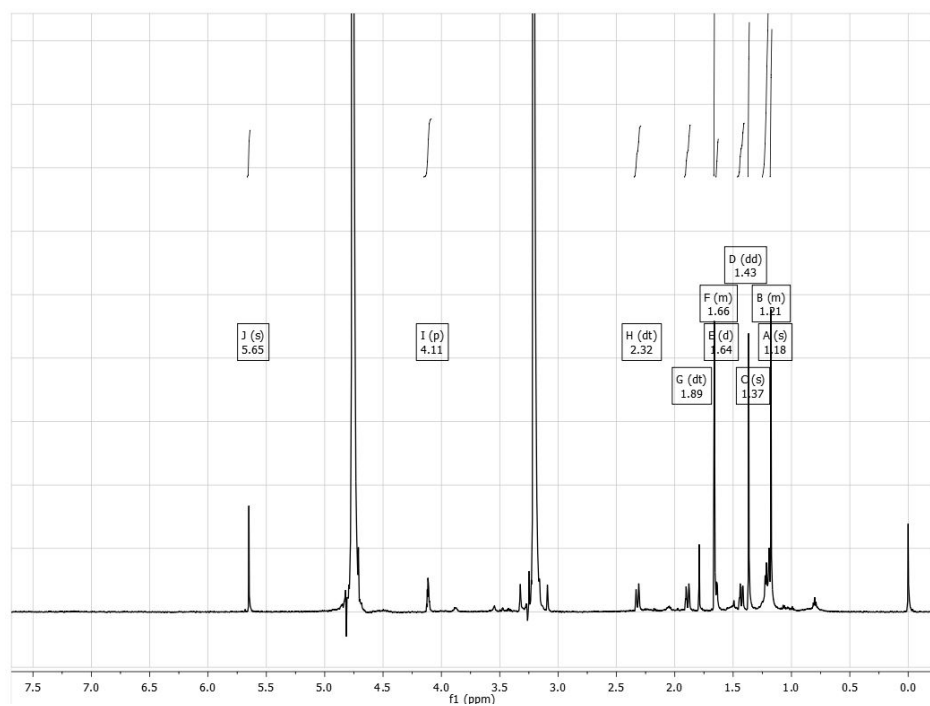


Figure S20. ^{13}C NMR spectrum of compound **6** (150 MHz, CD_3OD).

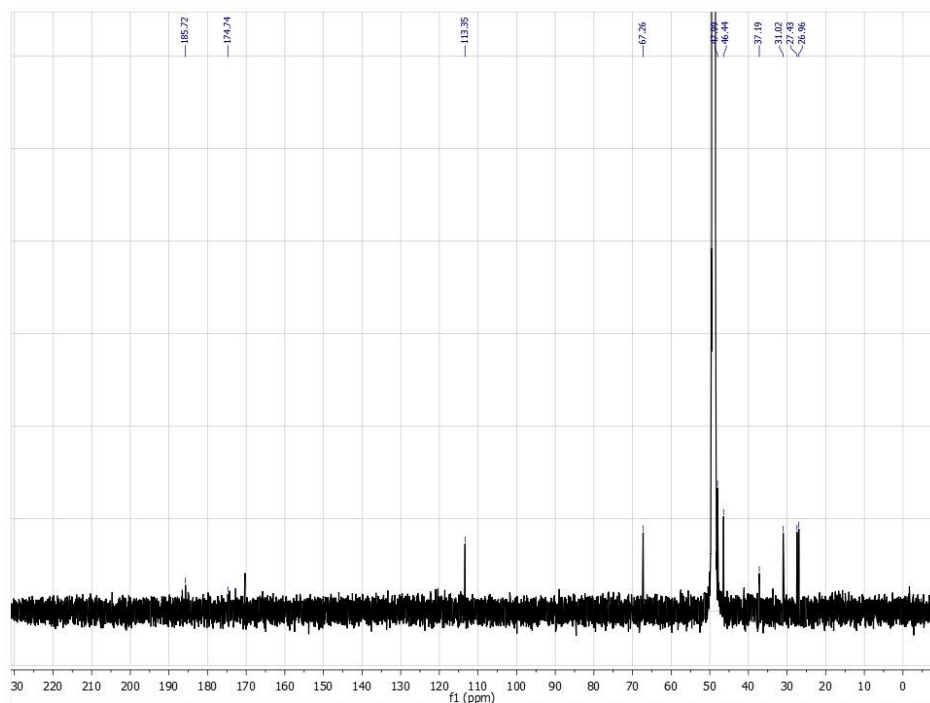


Figure S21. HR-ESIMS spectrum of compound **6**.



Figure S22. ^1H NMR spectrum of compound **7** (600 MHz, CD_3OD).

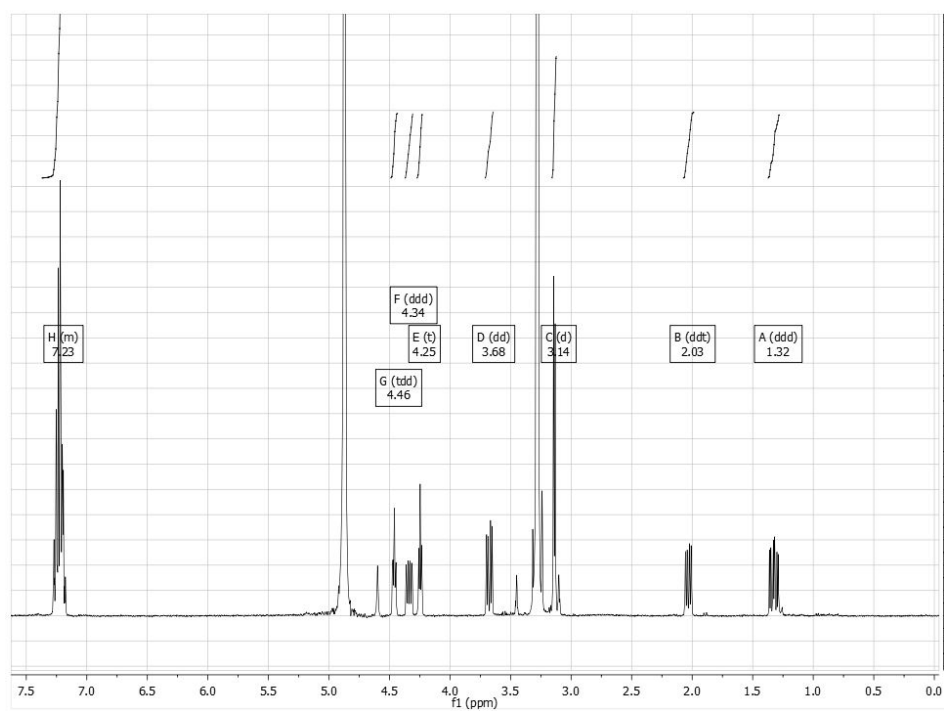


Figure S23. ^{13}C NMR spectrum of compound **7** (150 MHz, CD_3OD).

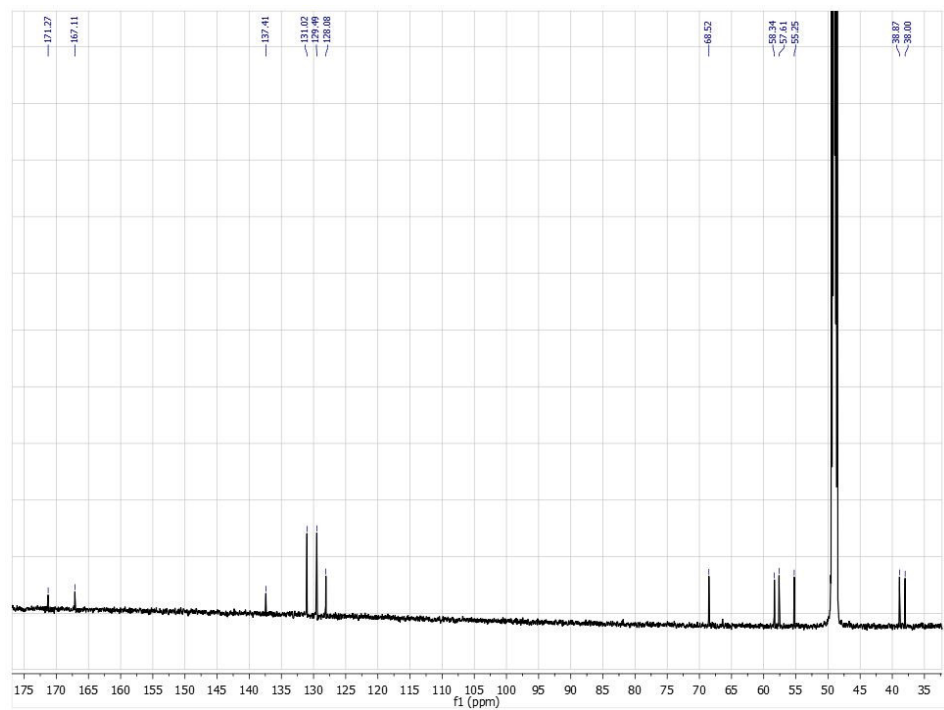


Figure S24. HR-ESIMS spectrum of compound 7.

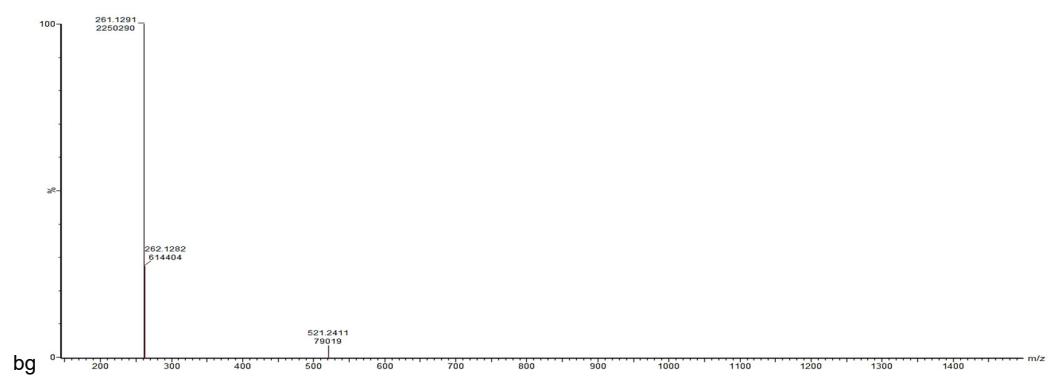


Figure S25. ^1H NMR spectrum of compound **8** (600 MHz, $\text{DMSO-}d_6$).

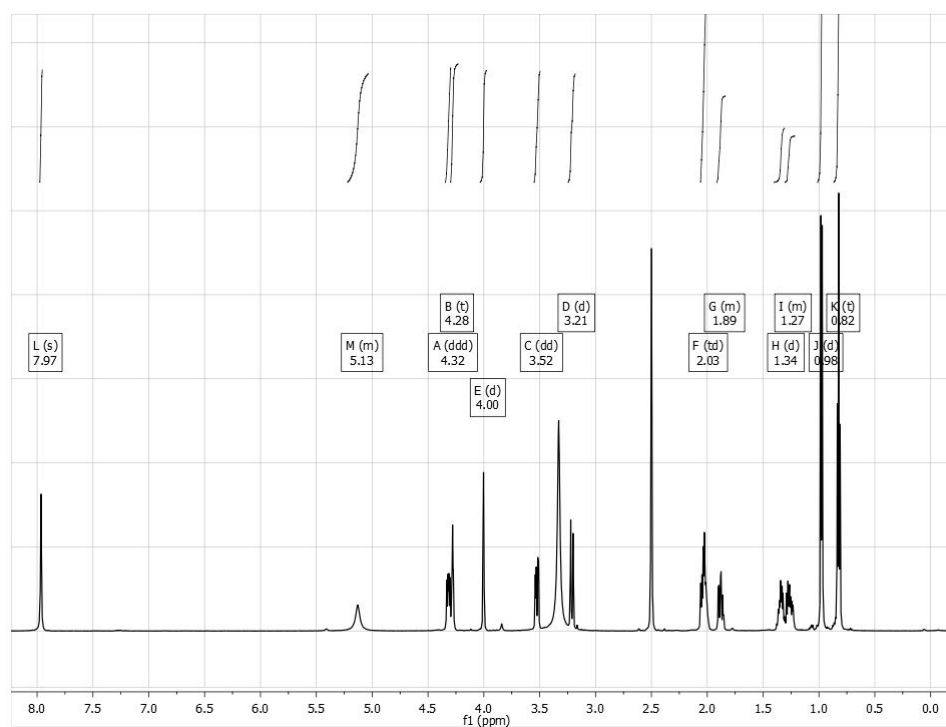


Figure S26. ^{13}C NMR spectrum of compound **8** (150 MHz, $\text{DMSO-}d_6$).

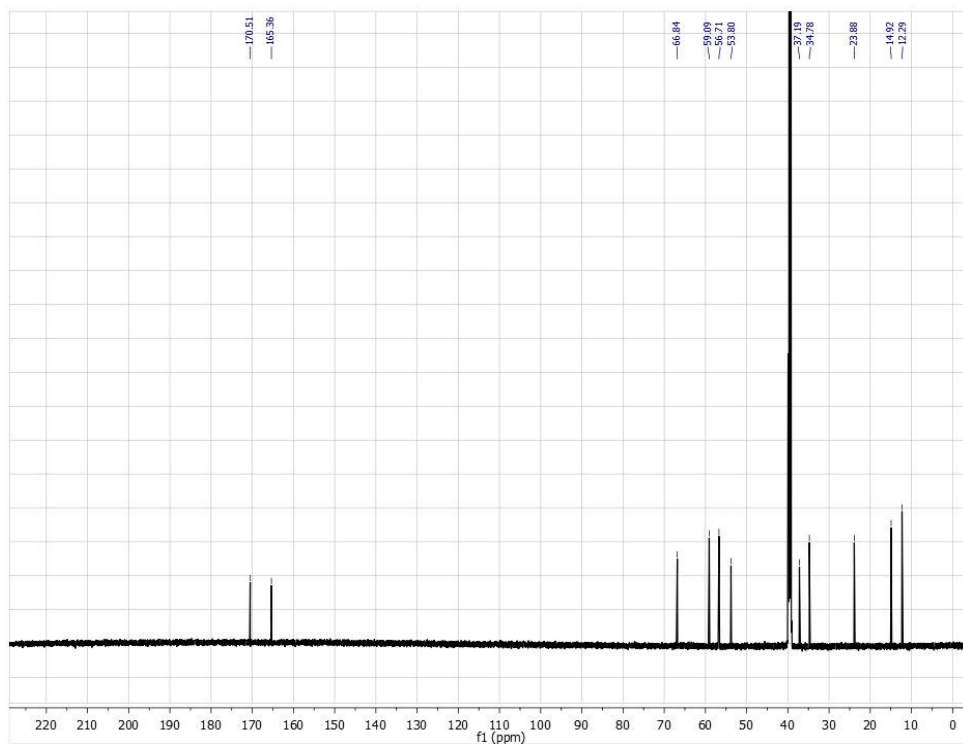


Figure S27. ^1H NMR spectrum of compound **8** (600 MHz, CD_3OD).

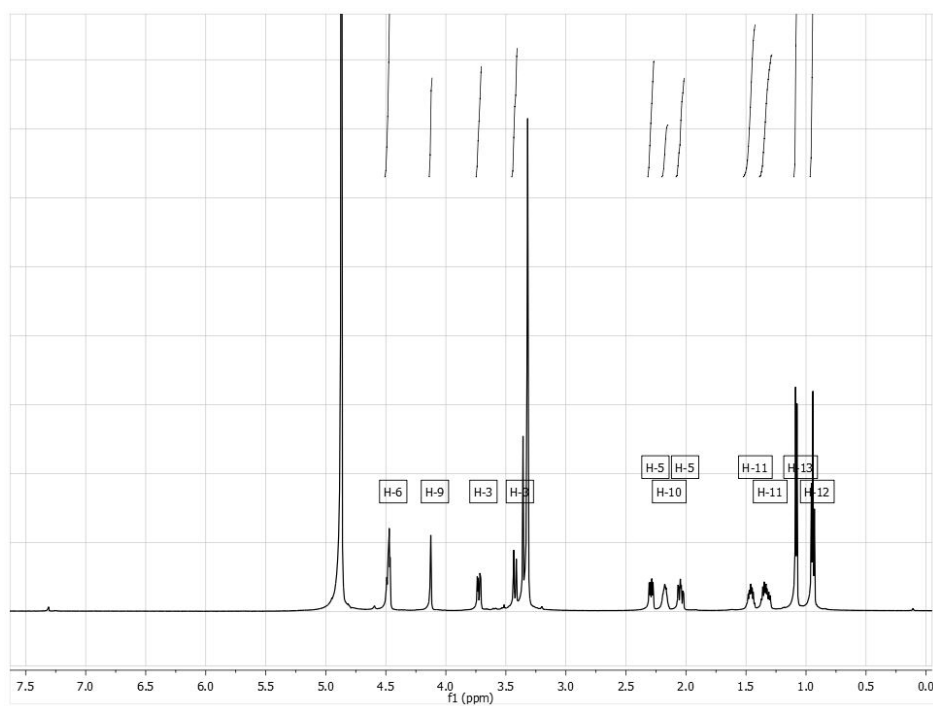


Figure S28. ^{13}C NMR spectrum of compound **8** (150 MHz, CD_3OD).

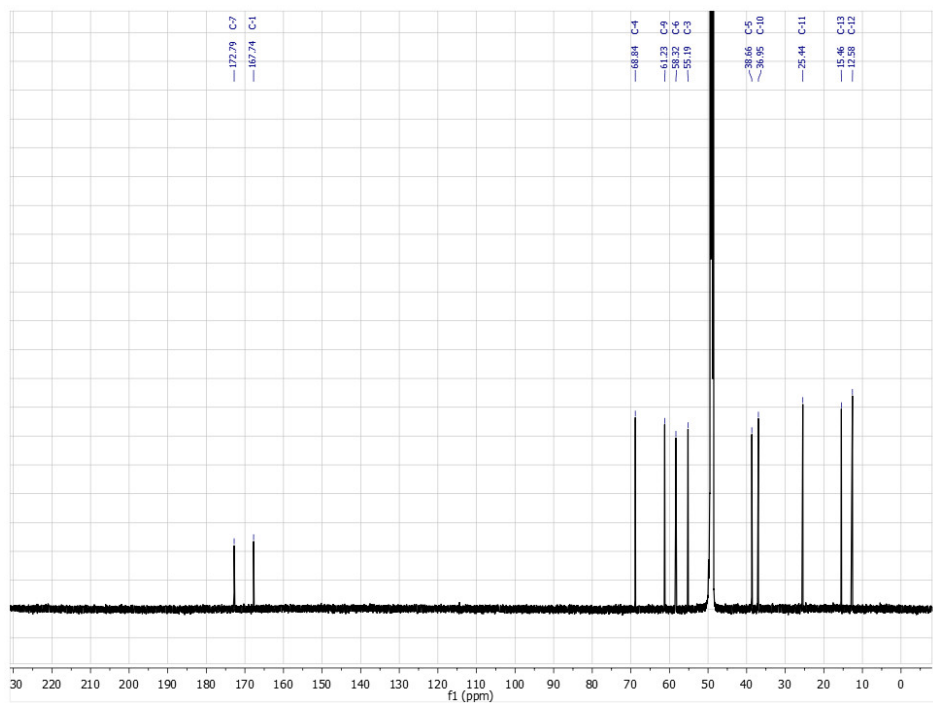


Figure S29. HSQC spectrum of compound **8** (150/600 MHz, CD₃OD).

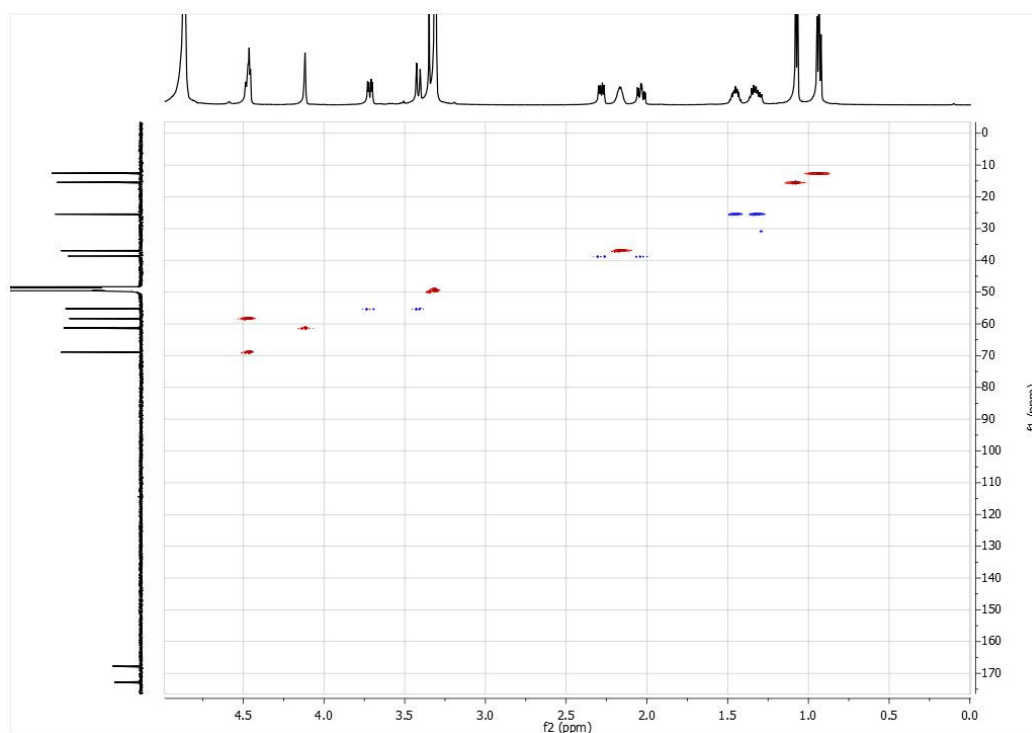


Figure S30. HMBC spectrum of compound **8** (150/600 MHz, CD₃OD).

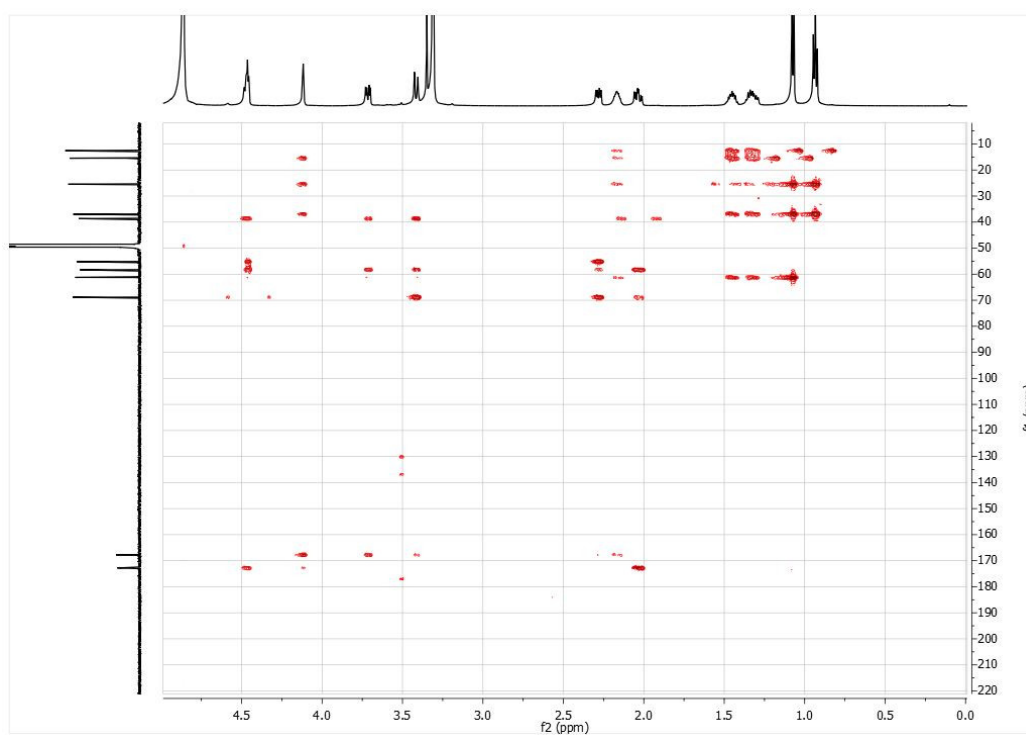


Figure S31. COSY spectrum of compound **8** (600 MHz, CD₃OD).

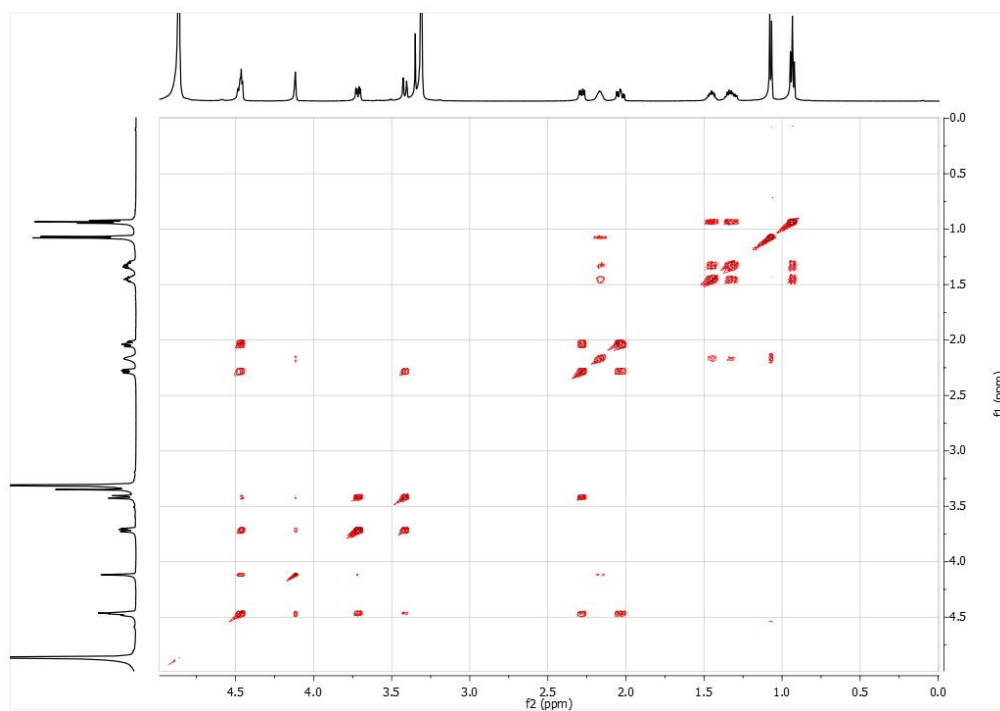


Figure S32. NOESY spectrum of compound **8** (600 MHz, CD₃OD).

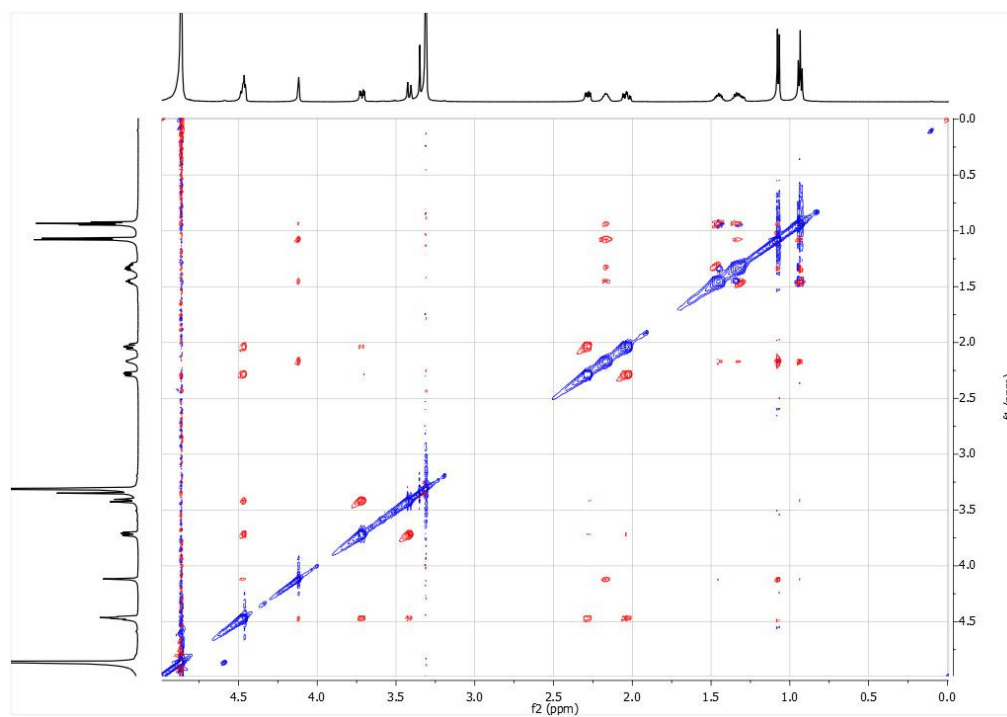


Figure S33. HR-ESIMS spectrum of compound **8**.



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